

**Amendment to the Specification:**

Please replace paragraphs [0009], [0010], [0045], [0053], [0073], [0078], [0083], [0085] and [0088] of the previously submitted Specification with the following corresponding corrected paragraphs.

**[0009]** Interesting hypothesis has been proposed that sporadic Alzheimer disease might be the brain type of non-insulin dependent diabetes mellitus (Hoyer, S. Is sporadic Alzheimer disease the brain ~~typ~~ type of non-insulin dependent diabetes mellitus? A challenging hypothesis. *J. Neural Transm.* 105, 415-422, 1998). It has been suggested that intracerebroventricular insulin enhances memory in a passive-avoidance task [Park, C. P., Seeley, R. J., Craft, S. and Woods S. C. (2000) Intracerebroventricular insulin enhances memory in a passive avoidance task. *Physiol. Behav.* 68, 509-514]. Insulin receptor density and tyrosine kinase activity in the sporadic Alzheimer's disease (SAD) or sporadic dementia of Alzheimer's type (SDAT) ~~was~~were known to be significantly decreased [Frolich, L., Blum-degen, D., Bernstein, H. G., Engelsberger, S., Humrich, J., Laufer, S., Muschner, D., Thalheimer, A., Turk, A., Hoyer, S., Zochling, R., Boissl, K. W., Jellinger, K., and Piederer, P. Brain insulin and insulin receptors in aging and sporadic Alzheimer's disease. *J. Neural Transm.* 105, 423-438, 1998]. Interestingly, tyrosine phosphorylation of the hippocampal insulin receptor has been shown to play an essential role in spatial memory formation [Zhao, W., Chen, H., Xu, H., Moore, E., Meiri, N., Quon, M. J., Alkon, D. L. (1999) Brain insulin receptors and spatial memory. *J. Biol. Chem.* 274, 34893-34902, 1999]. ~~Taken together, insulin receptor activators could be used for memory enhancement in addition to cholinesterase inhibitors.~~

**[0010]** Recently, it has been found that ERK (Extracellular signal-Regulated Kinase or MAPK) I and II, which are important downstream signaling mediators of the insulin receptor, are implicated in memory and learning [Thiels, E., Klann, E. Extracellular signal-regulated kinase, synaptic plasticity, and memory. *Rev. Neurosci.* 12, 327-345, 2001; Sweat J.D. The neuronal MAP kinase cascade: a biochemical signal integration system subserving

synaptic plasticity and memore. J. Neurochem. 76, 1-10, 2001]. It has been also demonstrated that rats subjected to avoidance learning showed significant and specific increases in the activated forms of ERK I and II in the rat hippocampus [Cammarota, M., Bevilaqua, L.R.M., Ardenghi, P., Paratcha, G., de Stein, M.L., Iaqueirido, I., Medina, J.H. Learning-associated activation of nuclear MAPK, CREB and Elk-1, along with Fos production, in the rat hoppocampus after a one-trial avoidance learning; abolition by NMDA receptor blockade. Mol. Brain Res. 76, 36-46, 2000]. Taken together, insulin receptor and ERK I/II activators could be used for memory enhancement in addition to cholinesterase inhibitors.

[0045] Cortical wedges were placed in a two-compartment bath and a greased (high vacuum silicon grease) barrier placed such that a high-resistance seal was formed between the two compartments. Oxygenated ACSF was perfused through the two compartments separately at 2 ml/min for at least 1 hr. Asiasari Radix (AR) extracts of 10 µg/ml (fractions 1, 2, 3 or 4) were perfused 10 min before the application of AMPA (40 µM) for 2 min: AR extracts and AMPA were applied to the cortical compartment. The DC potential between the two compartments was monitored via Ag/AgCl electrodes. The signal was amplified and analyzed with the aid of McLab software.

[0053] The cells were plated in 96 well plates at a density of  $1 \times 10^5$  cells/well. Cells were preincubated with AMPA (40 µM) and followed by the addition of AR fractions (10 µg/ml) for 24 hrs. MTT reagent (Sigma, USA) (5 mg/ml) was made in PBS (phosphate buffered saline) and filtered. The cells were then treated with MTT (final concentration, 0.5 mg/ml) and allowed to incubate for 3 hrs at 37°C. Cells containing active mitochondria form dark blue formazan by disintegrating tetrazolium ring. The culture media were removed and the cells were subjected to lysis in the presence of 100 F1 of DMSO and 10 F1 of Sorensen glycine buffer (0.1M glycine, 0.1M NaCl, pH 10.5). The absorbance was measured with spectrophotometer at 570 nm. The ratio of absorbance of experimental groups to control groups is expressed as % cell viability.

[0073] The test was basically performed according to the step-through method described by Jarvik and Kopp [Jarvik, M. E. and Kopp, R. An improved one-trial passive avoidance learning situation. *Pschol. Rep.* 21, 221-224, 1967]. The Gemini Avoidance System (SD Instruments) was used for this experiments. The apparatus consists of a two-compartment acrylic box with a lightened compartment connected to a darkened one by an automatic guillotine door. Fractions 1, 2 or 4 (10mg/kg/day, P.O.) was administered to mice once a day for three days and tested for the passive avoidance test. Mice were ~~were~~ placed in the lighted box for 300 sec. Then, the guillotine door was open. Mice, as soon as they entered the dark compartment, received a punishing electrical shock (0.3 mA, 1 sec). The latency times for entering the dark compartment were measured in the training test and after 24 hr in the retention test. The maximum entry latency allowed in the retention session was 500 sec.

[0078] Male Sprague Dawley rats were decapitated 60 minutes after the administration of AR extracts 10mg/kg and subjected to the isolation of hippocampus on 4°C. Hippocampal homogenates were prepared as described earlier with some modifications [Zhao, W., Chen, H., Xu, H., Moore, E., Meiri, N., Quon, M. J., Alkon, D. L., Insulin receptors and spatial memory. *J. Biol. Chem.* 274, 34893-34902, 1999]. The isolated hippocampus was resuspended with buffer A containing 50 mM Tris HCl, pH 7.4, 1 mM EDTA, 1 mM EGTA, 150 mM NaCl, 1% Triton X-100, 0.5 mM PMSF, 1 mM Na<sub>3</sub>VO<sub>4</sub>, 1ug/ml of leupeptin and aprotinin and subjected to homogenization with a Potter-Elvehjem homogenizer. The lysates were then spun at ~~1,000 x g for 5 min~~ 10,000 x g for 20 min and the supernatant were subjected to protein assay and saved at 70EC.

[0083] Equal amount of hippocampal proteins were applied to SDS polyacrylamide gel. Electrotransfer of proteins from the gels to nitrocellulose paper (Schleicher & Schuell) was carried out for 1 hr at 100 V (constant) as described by Towbin et al. [Towbin H., Staehelin, J., Gordon, J. Electric transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Aci. USA* 76, 4350-4354, 1979]. The

filter papers were preincubated for 1 hr at 23 °C with PBS containing 0.1% Tween 20 and 3% bovine serum albumin and washed with PBS containing 0.1% Tween 20 three times for 10 min each. The blots were probed with pTyr or pERK antibodies for 1 hr at 23 °C. The blots were then incubated with HRP-conjugated anti-rabbit IgG for 30 min and washed with PBS containing Tween 20 five times for 10 min each. The detection of immobilized specific antigens was carried out by ECL (NEN).

[0085] Since it has been suggested that tyrosine phosphorylation of the insulin receptors in hippocampus plays an important role in spatial memory, we have tested whether AR extracts have any effect on the tyrosine phosphorylation of hippocampal proteins. Tyrosine phosphorylation of a number of proteins with molecular sizes of ~180 kDa, 130 kDa, 95 kDa, 55 kDa and 42 kDa (Fig. 12). We further tested whether insulin receptor was tyrosine phosphorylated by the AR extracts. Insulin receptor phosphorylation was not detected under the basal condition whereas it was significantly stimulated by fraction 1 and fraction 2. The effect of fraction 1 was higher than that of fraction 2 (Fig. 13). In addition, the fraction 1, 2 and 4 of AR extracts has significantly stimulated ERK1 (44 kDa) and ERK2 (42 kDa) (Fig. 14). These results suggest that the fractions play an important role in memory and learning enhancement since activation of insulin receptor, ERK I and II are essential in memory and learning.

[0088] Male SD rats were dosed p.o. with vehicle or fractions of AR extract (10 mg/kg). The rats were decapitated after 90-60 min, brains rapidly removed, hippocampus-hippocampus and corpora striata dissected free, weighed and homogenized as described above. Cholinesterase activity was measured as described by Ellman et al [Ellman, G. L., Courtney, K. D., Andres, V., Featherstone, R. M. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 88-95.1961]. Briefly, 3 ml of buffer I (100 mM phosphate, pH 8.0), 0.2 ml of 75 mM acetylthiocholine iodide and 0.1 ml of buffered Ellman's reagent (DTNB 10 mM, NaHCO<sub>3</sub> 15 mM) were mixed and allowed to incubate for 10 min at 25EC. Then, 20 ml of enzyme sample was added and absorbance was

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measured at 30 sec intervals. The percent inhibition was calculated by comparison with the enzyme activity of the vehicle control group.